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The immune response of rainbow trout to *Flavobacterium psychrophilum* following immersion-challenge model with and without hydrogen peroxide pre-treatment

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The bacterial fish pathogen *Flavobacterium psychrophilum* is a major cause of mortality in farmed rainbow trout (*Oncorhynchus mykiss*) and other salmonid fish. The disease following infection is called bacterial coldwater disease (BCWD) or rainbow trout fry syndrome (RTFS). To our knowledge, no commercial vaccine is currently available and the disease is treated with antibiotics. Injection-based challenges with *F. psychrophilum* are standardized but the route of infection does not reflect a natural situation. Therefore, we established an immersion-based model investigating if hydrogen peroxide (H₂O₂) pre-treatment could elevate infection and mortality.

The model consisted of four groups: 1) Un-exposed control, 2) H₂O₂ exposure, 3) *F. psychrophilum* immersion and 4) H₂O₂ + *F. psychrophilum*. Pre-treatment with H₂O₂ increased mortality two-fold if fish also were exposed to *F. psychrophilum* after pretreatment. Tissue samples were taken from the involved groups 4 h, 48 h, 125 h and 192 h post-exposure and investigated for regulation of immune genes. Following genes were examined in the head kidney and gills by qPCR: IgT, IgM, CD8, CD4, MHC I, MHC II, IL-4/13A, TcR-β, IL-10, IL-6, IL-1β, IL-17, SAA and FoxP3.

A pro-inflammatory response was indicated, but only a weak indication of an adaptive response was recorded (most evident in the *F. psychrophilum* group). Further, pre-treatment with H₂O₂ affected the correlation gene expression and pathogen load in several cases. Morphological changes in the gill tissue were evaluated using hematoxylin and eosin stained tissue sections. Exposure to both H₂O₂ and *F. psychrophilum* intensified tissue damage and postponed healing. The results indicate that *F. psychrophilum* may have an immunosuppressive action and that environmental stress may be one of several factors playing a role in RTFS outbreaks.

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